

Appl. No. 09/699,667

### Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1 to 30 (canceled)

Claim 31 (currently amended): A method for cleaving a nucleic acid substrate with a nucleic acid enzyme comprising mixing said substrate with the nucleic acid enzyme of claim 1 ~~44~~.

Claim 32 (previously presented): The method of claim 31, wherein the substrate is composed of ribonucleotides.

Claim 33 (previously presented): The method of claim 31, wherein the substrate is composed of a mixture of ribonucleotides and deoxyribonucleotides.

Claims 34 to 43 (canceled)

Claim 44 (new): A nucleic acid enzyme capable of recognizing and cleaving a nucleic acid substrate, said nucleic acid enzyme which when bound to the substrate comprises:

(i) a first nucleotide sequence

5'-G<sup>1</sup>G<sup>2</sup>G<sup>3</sup>U<sup>4</sup>C<sup>5</sup>C<sup>6</sup>A<sup>13</sup>C<sup>14</sup>C<sup>15</sup>UC<sup>16</sup>C<sup>17</sup>UCGCG<sup>15</sup>G<sup>14</sup>U<sup>13</sup>N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>N<sup>6</sup>N<sup>7</sup>G<sup>17</sup>G<sup>16</sup>G<sup>7</sup>C<sup>8</sup>A<sup>9</sup>U<sup>10</sup>G<sup>11</sup>C<sup>12</sup>  
S<sup>1</sup>B<sup>1</sup>Y-3' (SEQ ID NO: 65); and

(ii) a second nucleotide sequence

5'-B<sup>2</sup>KS<sup>2</sup>G<sup>12</sup>C<sup>11</sup>A<sup>10</sup>U<sup>9</sup>G<sup>8</sup>G<sup>7</sup>CUAAGG<sup>6</sup>G<sup>5</sup>A<sup>4</sup>C<sup>3</sup>C<sup>2</sup>C<sup>1</sup>-3' (SEQ ID NO: 66);

wherein non-variable nucleotides having the same superscript form conventional Watson-Crick base pairs, except the two G<sup>7</sup> form a homopurine base pair;

S<sup>1</sup> and S<sup>2</sup> are each independently selected from the group consisting of G and C;

B<sup>1</sup> and B<sup>2</sup> are each independently selected from the group consisting of G, C, U and T;

K is selected from the group consisting of G, U and T;

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Y is selected from the group consisting of C, U and T;

S<sup>1</sup> and S<sup>2</sup> form a conventional Watson-Crick base pair;

Y and B<sup>2</sup> may form a conventional Watson-Crick base pair;

B<sup>1</sup> and K may form a conventional Watson-Crick base pair;

B<sup>1</sup>, K, Y and B<sup>2</sup> may together form a loop;

N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>N<sup>6</sup>N<sup>7</sup> forms a substrate binding region;

N<sup>1</sup>, N<sup>2</sup>, N<sup>3</sup>, N<sup>4</sup>, N<sup>5</sup> and N<sup>6</sup> are each a nucleotide which may be the same or different;

N<sup>7</sup> is U;

N<sup>7</sup> is capable of forming a wobble pair with the substrate;

N<sup>1</sup>, N<sup>2</sup>, N<sup>3</sup>, N<sup>5</sup> and N<sup>6</sup> are capable of forming conventional Watson-Crick base pairs with the substrate; and

N<sup>4</sup> is capable of forming a non-conventional Watson-Crick base pair and a conventional Watson-Crick base pair.

Claim 45 (new): The nucleic acid enzyme of claim 44, wherein said enzyme comprises a nucleotide sequence selected from the group consisting of:

(i) 5'-G<sup>1</sup>G<sup>2</sup>G<sup>3</sup>U<sup>4</sup>C<sup>5</sup>C<sup>6</sup>A<sup>13</sup>C<sup>14</sup>C<sup>15</sup>UC<sup>16</sup>C<sup>17</sup>UCGCG<sup>15</sup>G<sup>14</sup>U<sup>13</sup>N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>  
N<sup>6</sup>N<sup>7</sup>G<sup>17</sup>G<sup>16</sup>G<sup>7</sup>C<sup>8</sup>A<sup>9</sup>U<sup>10</sup>G<sup>11</sup>C<sup>12</sup>G<sup>18</sup>GCUUC<sup>18</sup>G<sup>12</sup>C<sup>11</sup>A<sup>10</sup>U<sup>9</sup>G<sup>8</sup>G<sup>7</sup>CUAAGG<sup>6</sup>G<sup>5</sup>A<sup>4</sup>C<sup>3</sup>C<sup>2</sup>C<sup>1</sup>-3'  
(SEQ ID NO: 61); and

(ii) 5'-G<sup>1</sup>G<sup>2</sup>G<sup>3</sup>U<sup>4</sup>C<sup>5</sup>C<sup>6</sup>A<sup>13</sup>C<sup>14</sup>C<sup>15</sup>UC<sup>16</sup>C<sup>17</sup>UCGCG<sup>15</sup>G<sup>14</sup>U<sup>13</sup>N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>  
N<sup>6</sup>N<sup>7</sup>G<sup>17</sup>G<sup>16</sup>G<sup>7</sup>C<sup>8</sup>A<sup>9</sup>U<sup>10</sup>G<sup>11</sup>C<sup>12</sup>C<sup>18</sup>UUCGG<sup>18</sup>G<sup>12</sup>C<sup>11</sup>A<sup>10</sup>U<sup>9</sup>G<sup>8</sup>G<sup>7</sup>CUAAGG<sup>6</sup>G<sup>5</sup>A<sup>4</sup>C<sup>3</sup>C<sup>2</sup>C<sup>1</sup>-3'  
(SEQ ID NO: 62).

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Claim 46 (new): The nucleic acid enzyme of claim 44, wherein said first nucleotide sequence is 5'-G<sup>1</sup>G<sup>2</sup>G<sup>3</sup>U<sup>4</sup>C<sup>5</sup>C<sup>6</sup>A<sup>13</sup>C<sup>14</sup>C<sup>15</sup>UC<sup>16</sup>C<sup>17</sup>UCGCG<sup>15</sup>G<sup>14</sup>U<sup>13</sup>N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>N<sup>6</sup>N<sup>7</sup>G<sup>17</sup>G<sup>16</sup>G<sup>7</sup>C<sup>8</sup>A<sup>9</sup>U<sup>10</sup>G<sup>11</sup>C<sup>12</sup>G<sup>18</sup>C<sup>19</sup>C<sup>20</sup>-3' (SEQ ID NO: 63) and said second nucleotide sequence is 5'-G<sup>20</sup>G<sup>19</sup>C<sup>18</sup>G<sup>12</sup>C<sup>11</sup>A<sup>10</sup>U<sup>9</sup>G<sup>8</sup>G<sup>7</sup>CUAAGG<sup>6</sup>G<sup>5</sup>A<sup>4</sup>C<sup>3</sup>C<sup>2</sup>C<sup>1</sup>-3' (SEQ ID NO: 64).

Claim 47 (new): The nucleic acid of claim 44, wherein N<sup>1</sup>N<sup>2</sup>N<sup>3</sup>N<sup>4</sup>N<sup>5</sup>N<sup>6</sup>N<sup>7</sup> is selected from the group consisting of:

- (i) CCGACCU;
- (ii) CCCAGCU;
- (iii) GGGUAUUAU;
- (iv) CCGGCCU;
- (v) CCGGCCU;
- (vi) CCGUCCU;
- (vii) CCGAACU;
- (viii) CCGAGCU;
- (ix) CCGAUCU;
- (x) CCUCUUU;
- (xi) CCUUGUU;
- (xii) UGUUCUU;
- (xiii) GGGGUUU;
- (xiv) UCCGCCU;
- (xv) GGACUCU;

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(xvi) UCGACUU; and

(xvii) GCCACCU.

Claim 48 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is derived from hepatitis delta virus.

Claim 49 (new): The nucleic acid enzyme of claim 44, wherein the substrate is composed of ribonucleotides.

Claim 50 (new): The nucleic acid enzyme of claim 44, wherein the substrate is composed of a mixture of ribonucleotides and deoxyribonucleotides.

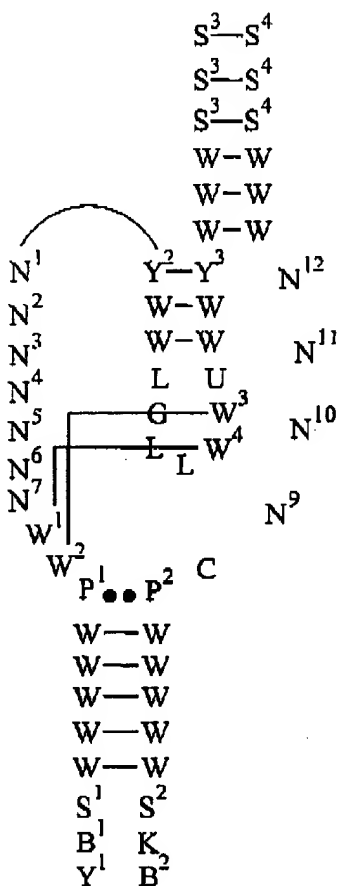
Claim 51 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is composed of ribonucleotides.

Claim 52 (new): The nucleic acid enzyme of claim 44, wherein the enzyme is composed of a mixture of ribonucleotides and deoxyribonucleotides.

Claim 53 (new) The nucleic acid enzyme of claim 44, wherein the nucleotide residue of said substrate directly 5' to the cleavage site does not form a base pair with said enzyme.

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Claim 54 (new): A nucleic acid enzyme capable of recognizing and cleaving a nucleic acid substrate at a cleavage site, which when bound to the substrate comprises:



$Y^2$  and  $Y^3$  together form a Watson-Crick base pair and are each independently selected from U, A and T;

$S^3$  and  $S^4$  together form a Watson-Crick base pair and are each independently selected from U, A, G and C;

$N^9$ ,  $N^{10}$ ,  $N^{11}$  and  $N^{12}$  are nucleotides none of which form part of a Watson-Crick base pair;

$P^1$  and  $P^2$  are the same purine nucleotide forming a homopurine base pair;

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$W$ ,  $W^1$ ,  $W^2$ ,  $W^3$  and  $W^4$  are each independently selected from any nucleotide that is part of a Watson-Crick base pair;

$W^1$  and  $W^4$  form a Watson-Crick base pair;

$W^2$  and  $W^3$  form a Watson-Crick base pair;

$L$  are each independently selected from any nucleotide, each  $L$  is single stranded and does not form a Watson-Crick base pair;

one to two  $L$  may be absent;

$Y^1$  and  $B^2$  may be joined to form a loop or may not form a loop but may form a Watson-Crick base pair;

$S^1$  and  $S^2$  may form a Watson-Crick base pair;

$B^1$  and  $K$  may form a Watson-Crick base pair;

$B^1$ ,  $K$ ,  $Y^1$  and  $B^2$  may together form a loop;

wherein  $S^1$  and  $S^2$  are independently selected from the group consisting of  $G$  and  $C$ ;

$B^1$  and  $B^2$  are each independently selected from the group consisting of  $G$ ,  $C$ ,  $A$ ,  $U$  and  $T$ ;

$K$  is selected from the group consisting of  $C$ ,  $A$ ,  $G$ ,  $U$  and  $T$ ;

$Y^1$  is selected from the group consisting of  $G$ ,  $A$ ,  $C$ ,  $U$  and  $T$ ;

$N^1N^2N^3N^4N^5N^6N^7$  forms a substrate binding region;

$N^1$ ,  $N^2$ ,  $N^3$ ,  $N^4$ ,  $N^5$  and  $N^6$  are each a nucleotide which may be the same or different;

$N^7$  is  $U$ ;

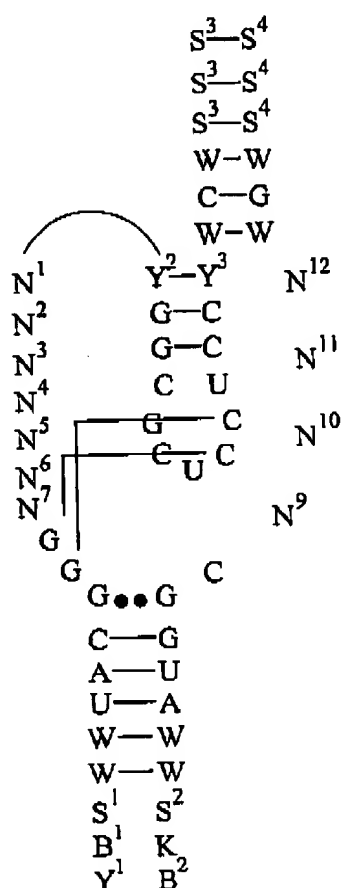
$N^7$  is capable of forming a wobble pair with the substrate;

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$N^1$ ,  $N^2$ ,  $N^3$ ,  $N^5$  and  $N^6$  are capable of forming conventional Watson-Crick base pairs with the substrate; and

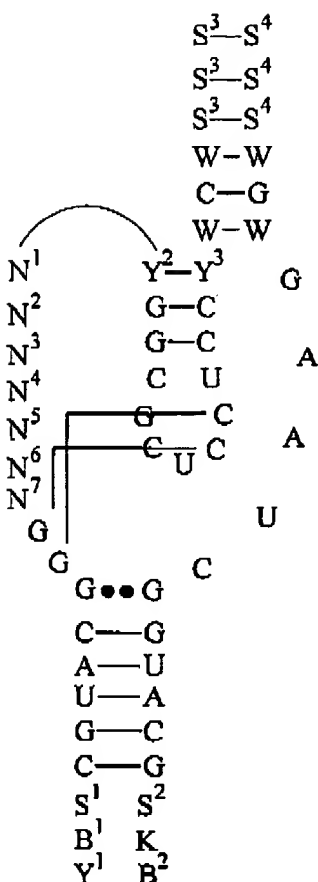
$N^4$  is capable of forming a non-conventional Watson-Crick base pair and a conventional Watson-Crick base pair.

Claim 55 (new): The nucleic acid enzyme of claim 54, wherein the enzyme is further defined as follows:



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**Claim 56 (new):** The nucleic acid enzyme of claim 54, wherein the enzyme is further defined as follows:



**Claim 57 (new):** The nucleic acid enzyme of claim 44, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 58 (new): The nucleic acid enzyme of claim 45, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.



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Claim 59 (new): The nucleic acid enzyme of claim 46, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 60 (new): The nucleic acid enzyme of claim 54, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 61 (new): The nucleic acid enzyme of claim 55, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.

Claim 62 (new): The nucleic acid enzyme of claim 56, wherein the enzyme is adapted to bind to the substrate, the substrate having the following characteristics: nucleotide residues directly 5' to the cleavage site do not interact with the enzyme and the nucleotide residue directly 3' to the cleavage site is G, forming a GU wobble pair with the enzyme.